according to standard procedures to give DMT-A_{SP}-T-CPG oligomerization with chirally pure Sp dimer units at the termini.

g. Oligonucleotide Synthesis

[0218] The oligonucleotide having the sequence T*GC ATC CCC CAG GCC ACC A*T SEQ ID NO: 22 is synthesized, where T*G and A*T represent chiral Sp dimer blocks described above. DMT-A_{SP}-T-CPG is taken in the synthesis column and the next 16b residues are built using standard phosphorothioate protocols and 3H-1,2-benzodithiol-3-one 1,1 dioxide as the sulfurizing agent. After building this 18 mer unit followed by final detritylation, the chiral Sp dimer phosphoramidite of 5'-DMT-T_{SP}-G amidite is coupled to give the desired antisense oligonucleotide. This compound is then deprotected in 30% NH_4OH over 16 hours and the oligomer purified in HPLC and desalted in Sephader G-25 column. The final oligomer has Sp configuration at the 5'-terminus and 3'-terminus and the interior has diastereomeric mixture of Rp and Sp configurations.

EXAMPLE 60

Evaluation of in vivo stability of MMI capped oligonucleotides

mouse experiment procedures

[0219] For each oligonucleotide tested, 9 male BALB/c mice (Charles River, Wilmington, MA), weighing about 25 g was used (Crooke *et al., J. Pharmacol. Exp. Ther.*, 1996, *277*, 923). Following a 1-week acclimation, mice received a single tail vein injection of oligonucleotide (5 mg/kg) administered in phosphate buffered saline (PBS), pH 7.0 One retro-orbital bleed (either 0.25, 0.5, 2 or 4 lv post dose) and a terminal bleed (either 1, 3, 8 or 24 h post dose) were collected from each group. The terminal bleed (approximately 0.6-0.8 mL) was collected by cardiac puncture following ketamine/xylazine anesthesia. The blood was transferred to an EDTA-coated collection tube and centrifuged to obtain plasma. At termination, the liver and kidneys were collected from each mouse. Plasma and tissues homogenates were used for analysis for determination of intact oligonucleotide content by CGE. All samples were

immediately frozen on dry ice after collection and stored at -80 °C until analysis.

[0220] The capillary gel electrophoretic analysis indicated the relative nuclease resistance of MMI capped oligomers compared to ISIS 3082 (uniform 2'-deoxy phosphorothioate). Because of the resistance of MMI linkage to nucleases, the compound 16314 was found to be stable in plasma while 3082 was not. However, in kidney and liver, the compound 16314 also showed certain amount of degradation. This implied that while 3'-exonuclease is important in plasma, 5'-exonucleases or endonucleases may be active in tissues. To distinguish between these two possibilities, the data from 16315 was analyzed. In plasma as well as in tissues, (liver and kidney) the compound was stable in various time points. (1, 3 and 24 hrs.). The fact that no degradation was detected proved that 5'-exonucleases and 3'-exonuclease are prevalent in tissues and endonucleases are not active. Furthermore, a single linkage (MMI or Sp thioate linkage) is sufficient as a gatekeeper against nucleases.

Control of ICAM-1 Expression Cells and Reagents

[0221] The bEnd.3 cell line, a brain endothelioma, was the kind gift of Dr. Werner Risau (Max-Planck Institute). Opti-MEM, trypsin-EDTA and DMEM with high glucose were purchased from Gibco-BRL (Grand Island, NY). Dulbecco's PBS was purchased from Irvine Scientific (Irvine, CA). Sterile, 12 well tissue culture plates and Facsflow solution were purchased from Becton Dickinson (Mansfield, MA). Ultrapure formaldehyde was purchased from Polysciences (Warrington, PA). Recombinant human TNF-a was purchased from R&D Systems (Minneapolis, MN). Mouse interferon-γ was purchased from Genzyme (Cambridge, MA). Fraction V, BSA was purchased from Sigma (St. Louis, MO). The mouse ICAM-1-PE, VCAM-1-FITC, hamster IgG-FITC and rat IgG_{2a}-PE antibodies were purchased from Pharmingen (San Diego, CA). Zeta-Probe nylon blotting membrane was purchased from Bio-Rad (Richmond, CA). QuickHyb solution was purchased from Stratagene (La Jolla, CA). A cDNA labeling kit, Prime-a-Gene, was purchased from ProMega (Madison, WI). NAP-5 columns were purchased from Pharmacia (Uppsala, Sweden).

Oligonucleotide Treatment

[0222] Cells were grown to approximately 75 % confluency in 12 well plates with DMEM containing 4.5 g/L glucose and 10 % FBS. Cells were washed 3 times with Opti-MEM pre-warmed to 37 °C. Oligonucleotide was premixed with Opti-MEM, serially diluted to desired concentrations and transferred onto washed cells for a 4 hour incubation at 37 °C. Media was removed and replaced with normal growth media with or without 5 ng/mL TNF-α and 200 U/mL interferon-γ, incubated for 2 hours for northern blot analysis of mRNA or overnight for flow cytometric analysis of cell surface protein expression.

Flow Cytometry

[0223] After oligonucleotide treatment, cells were detached from the plates with a short treatment of trypsin-EDTA (1-2 min.). Cells were transferred to 12x75 mm polystyrene tubes and washed with 2% BSA, 0.2% sodium azide in D-PBS at 4 °C. Cells were centrifuged at 1000 rpm in a Beckman GPR centrifuge and the supernatant was then decanted. ICAM-1, VCAM-1 and the control antibodies were added at 1 ug/mL in 0.3 mL of the above buffer. Antibodies were incubated with the cells for 30 minutes at 4 °C in the dark, under gentle agitation. Cells were washed again as above and then resuspended in 0.3 mL of FacsFlow buffer with 0.5 % ultrapure formaldehyde. Cells were analyzed on a Becton Dickinson FACScan. Results are expressed as percentage of control expression, which was calculated as follows: [((CAM expression for oligonucleotide-treated cytokine induced cells) - (basal CAM expression))/((cytokine-induced CAM expression) - (basal CAM expression))] X 100. For the experiments involving cationic lipids, both basal and cytokine-treated control cells were pretreated with Lipofectin for 4 hours in the absence of oligonucleotides.

[0224] The results reveal the following: 1) Isis 3082 showed an expected dose response (25-200 nM); 2) Isis 13001 lost its ability to inhibit ICAM-1 expression as expected from a mismatch compound, thus proving an antisense mechanism; 3) 3'-MMI capped oligomer 16314 improved the activity of 3082, and at 200 nM concentration, nearly twice as active as 3082; 4) 5'- and 3'- MMI capped oligomer is the most potent compound and it is nearly 4 to 5 times more